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Di(2-ethylhexyl) Adipate: Condensation of the Carcinogenesis Bioassay Technical Report*

Di(2-ethylhexyl) adipate [bis(2-ethylhexyl) adipate, DEHA, octyl adipate, dioctyl adipate, DOA, CAS No. 103-23-1], a plasticizer added to vinyl plastics to give low temperature flexibility, is dispersed (not bound) in the polymer chain matrix. Approved by the U.S. Food and Drug Administration for use in plastics that may contact nonfatty, nonalcoholic foods (not to exceed 24% by weight of the plastic polymer), DEHA has wide use in vinyl packaging film for refrigerated and frozen food products. Other products containing DEHA include electric wire insulation, garden hoses, vinyl coated fabrics for automotive and upholstery use, synthetic rubber, base oils for hydraulic fluids, and, among others, polyvinyl tubing for hemodialysis. For these uses 44 million pounds were produced in 1978.

DEHA was selected as a representative of the adipate class of plasticizers, because it is structurally related to di(2-ethylhexyl) phthalate, because human exposure is widespread, and because no carcinogenesis studies had been done. Testing was initiated by the Carcinogenesis Testing Program, National Cancer Institute (now part of the National Institute of Environmental Health Sciences/National Toxicology Program).

Methods

Male and female inbred Fischer 344 rats and male and female hybrid $B6C3F_1$ mice, obtained from the Frederick Cancer Research Center, were used in this bioassay. For 103 consecutive weeks all groups received powdered Wayne Lab Blox. Treated groups were fed this diet containing 12,000 ppm or 25,000 ppm di(2-ethylhexyl) adipate (> 98% pure).

This carcinogenesis bioassay was conducted between April 1977 and May 1979 at EG&G Mason Research Institute under a subcontract to Tracor Jitco (prime contractor for the testing program).

All animals that died during the study or that were killed at the end of the exposure period were subjected to a gross necropsy and a complete histopathological examination. Statistical analyses comparing survival and numbers of animals with specific site tumors were done with trend tests and pairwise comparisons (*1-4*). The study design conformed to the NCI Guidelines for carcinogen bioassay (*5*).

Results

Mean body weights of high dose male and female rats and treated male and female mice were lower than the corresponding controls. Survival was comparable among all groups of mice and male rats; survival in the female control rats was reduced relative to the treated groups (29/50 control, 39/50 low dose, 44/50 high dose).

The number of DEHA-treated rats with specific site tumors did not differ significantly from those found in controls. Table 1 lists those primary tumors occurring in at least three animals of any one group.

Liver tumors occurred with significantly higher frequencies in treated male and female mice than in controls (Table 2). Other specific site tumors were not significantly increased in treated mice when compared to controls (Table 3).

Discussion

In male rats the positive dose-related trend ($p = 0.010$) and the statistically significant ($p < 0.05$) increase in interstitial cell testicular tumors in the

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Table 1. Primary tumors in male and female F344 rats fed diets containing di(2-ethylhexyl) adipate.

	Male			Female		
Tumor	Control	12,000 ppm	25,000 ppm	Control	12,000 ppm	25,000 ppm
Adrenal cortical adenoma	1/48	1/50	0/50	3/50	0/50	0/48
Adrenal pheochromocytoma	6/48	3/50	1/50	1/50	2/50	3/48
Hepatocellular carcinoma	0/49	1/50	2/50	0/49	1/50	0/50
Hepatocellular neoplastic nodule	2/49	1/50	0/50	0/49	2/50	1/50
Leukemia	9/49	11/50	8/50	12/50	5/50	10/50
Mammary gland	1/49	2/50	0/50	14/50	6/50	3/50
Mesothelioma	1/49	1/50	3/50	0/50	0/50	0/50
Pituitary adenoma or carcinoma	4/44	3/47	3/44	21/47	25/48	18/49
Preputial gland carcinoma	1/49	4/50	1/50[a]	–	–	–
Skin squamous cell papilloma	1/49	4/50	1/50	0/50	0/50	0/50
Subcutaneous fibroma	4/49	1/50[b]	2/50	0/50[b]	1/50	0/50[c]
Testicular interstitial cell	43/49	47/50	49/49	–	–	–
Thyroid C-cell carcinoma or adenoma	3/49	4/49	1/46	4/50	0/50	3/47
Uterine endometrial stromal polyp	–	–	–	11/50	10/50	13/50

[b]One other animal had a subcutaneous febrosarcoma.
[c]One female had a subcutaneous fibroadenoma.

Table 2. Primary liver tumor increases in male and female B6C3F$_1$ mice fed diets containing di(2-ethylhexyl) adipate.

	Males			Females		
Hepatocellular tumor	Control	12,000 ppm	25,000 ppm	Control	12,000 ppm	25,000 ppm
Carcinoma[a]	7/50	12/49	12/49[b]	1/50	14/50[c]	12/49[d]
Adenoma[e]	6/50	8/49	15/49[f]	2/50	5/50	6/49
Carcinoma or adenoma[g]	13/50	20/49	27/49[d]	3/50	19/50[c]	18/49[c]

[a]Significant dose-related trend for females ($p < 0.005$).
[b]One other male had a neoplasm, NOS (not otherwise specified).
[c]Significantly greater than controls ($p < 0.001$).
[d]Significantly greater than controls ($p < 0.005$).
[e]Significant dose-related trend for males ($p < 0.05$).
[f]Significantly greater than controls ($p < 0.05$).
[g]Significant dose-related trend for males and females ($p < 0.005$).

Table 3. Primary tumors in male and female B6C3F$_1$ mice fed diets containing di(2-ethylhexyl) adipate.

	Males			Females		
Tumor	Control	12,000 ppm	25,000 ppm	Control	12,000 ppm	25,000 ppm
Circulatory system: angiosarcoma, hemangioma, hemangiosarcoma	2/50	2/50	1/50	3/50	3/50	3/49
Lymphoma	16/50	7/50	6/49	23/50	14/50	7/49
Lung alveolar/bronchiolar adenoma	8/50	9/49	3/49	5/49[a]	1/49	3/48
Pituitary adenoma	0/40	0/31	0/36	8/39	6/37	0/39
Subcutaneous fibroma or fibrosarcoma or sarcoma, NOS	5/50	3/50	0/49	2/50[b]	0/50	2/50

[a]One other female rat had an alveolar/bronchiolar carcinoma.
[b]One other female rat had a neoplasm, NOS (not otherwise specified).

high dose group were not considered compound related since most (> 80%) aging F344 male rats have this lesion.

Significant negative trends were obtained for male rats with adrenal gland pheochromocytomas ($p < 0.05$) and for female rats with adrenal cortical adenomas ($p < 0.05$) and with mammary gland fibroadenomas ($p = 0.001$).

Hepatocellular carcinomas were increased in both low and high dose female mice; hepatocellular adenomas or carcinomas combined occurred in high dose mice of either sex and in low dose female mice at incidences that were dose related and significantly higher than those in the controls. The time to observation of hepatocellular adenomas or carcinomas in the dosed female mice, but not in dosed male

mice, was significantly shorter than the time to observation of these tumors in the controls. Because the increase in liver tumors in males reflects only an increase in adenomas for the high dose group and because the time to observation of tumors in dosed groups as compared with the control group was not significantly different, the association of liver tumors in the males with administration of di(2-ethylhexyl) adipate is not considered conclusive.

Hepatocellular adenoma compressed the adjacent liver tissue. Cells in the adenoma were large. Cytoplasm of the cells was acidophilic or vacuolated and nuclei were hyperchromatic. Hepatocellular carcinoma involved a part or an entire lobe of the liver. The lobular architecture was distorted and cell plates were two or more cells thick, forming trabeculae. Cellular pleomorphism was apparent. The nuclei had coarse chromatin, and the nucleoli were prominent. Both normal and abnormal mitotic figures were numerous. Areas of necrosis and mineralization were common in the large tumors.

Hepatocellular carcinoma metastasized to the lung in 14 male mice (control, 5; low dose, 4; high dose, 5) and in 11 female mice (low dose, 6; high dose, 5). In all cases, the primary liver tumors were of the trabecular type. The other sites of metastases were the kidney, adrenal, and lymph nodes in dosed female mice.

The historical incidence of liver tumors in male $B6C3F_1$ mice at this laboratory is: adenomas, 35/398 (9%, range 0-16%); carcinomas, 86/398 (22%, range 14-30%); combined tumors, 116/398 (29%, range 18-36%). The historical incidence of tumors in female $B6C3F_1$ mice at this laboratory is: adenomas, 18/397 (5%, range 0-18%); carcinomas, 14/397 (4%, range 0-8%); combined tumors, 31/397 (8%, range 2-20%).

Negative trends were calculated for male ($p = 0.010$) and for female ($p = 0.001$) mice with lymphomas; the number of mice in each treated group with lymphoma was significantly less ($p < 0.05$) than the corresponding controls. This interesting phenomenon of decreases in leukemias/lymphomas concomitant with increases in hepatocellular neoplasms is receiving considerable research attention from the National Toxicology Program (*6*).

Di(2-ethylhexyl) adipate was not mutagenic for *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98, and TA 100, with and without activation, or for *Saccharomyces cerevisiae* (*7*). Di(2-ethylhexyl) adipate is teratogenic for Sprague-Dawley rats (*8*) and causes dominant lethal mutations in ICR mice (*9*).

In conclusion and under the conditions of this bioassay, di(2-ethylhexyl) adipate was not carcinogenic for F344 rats of either sex. Di(2-ethylhexyl) adipate was carcinogenic for female $B6C3F_1$ mice causing increased numbers of female mice with hepatocellular carcinomas, and was probably carcinogenic for male $B6C3F_1$ mice, causing increased numbers of male mice with hepatocellular adenomas as well as inducing increased numbers of male mice with combined hepatocellular adenomas or carcinomas (*10*).

REFERENCES

1. Armitage, P. Statistical Methods in Medical Research. John Wiley & Sons, Inc., New York, 1971, pp. 362-365.
2. Cox, D. R. Analysis of Binary Data. Methuen & Co., Ltd., London, 1970, pp. 48-52.
3. Gart, J. J. The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39: 148-169 (1971).
4. Tarone, R. E. Tests for trend in life table analysis. Biometrika 62: 697-682, 1975.
5. Sontag, J. M., Page, N. P., and Saffiotti, U. Guidelines for Carcinogen Bioassay in Small Rodents (National Cancer Institute Carcinogenesis Technical Report Series No. 1), DHEW Publication No. 76-801, Washington, D.C., 1976.
6. Haseman, J. K. Patterns of tumor incidence in two-year cancer bioassay feeding studies in Fischer (F344) rats. Fund. Appl. Toxicol. In press.
7. Simmon, V., Kauhanen, K., and Tardiff, R. Mutagenic activity of chemicals identified in drinking water. Dev. Toxicol. Environ. Sci. 2: 249-258 (1977).
8. Singh, A., Lawrence, W., and Autian, J. Embryonic-fetal toxicity and teratogenic effects of adipic acid esters in rats. J. Pharm. Sci. 62: 1596-1600 (1973).
9. Singh, A., Lawrence, W., and Autian, J. Dominant lethal mutations and antifertility effects of di-2-ethylhexyl adipate and diethyl adipate in male mice. Toxicol. Appl. Pharmacol. 32: 566-576, 1975.
10. NCI/NTP. Carcinogenesis Bioassay of Di(2-ethylhexyl)adipate (TR 212). National Cancer Institute/National Toxicology Program, U.S. Department of Health and Human Services, 1982, 121 pp.